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Variation among hexaploid *Paspalum dilatatum* Poir. regenerants from tissue culture

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Abstract. The common biotype of *Paspalum dilatatum* Poir. (dallisgrass) is a pentaploid obligate apomict and efforts to improve the grass have not been successful because of its asexual reproduction and irregular meiosis. An apomictic hexaploid biotype, known as Uruguayan dallisgrass, is a new source of genetic variation that may be useful in improving dallisgrass. As with common dallisgrass, improvement of this biotype via conventional breeding methods is difficult because of its apomictic reproduction. However, the use of tissue culture to produce somaclonal variation in the Uruguayan biotype has not been reported, and may offer a means for improving the species. The objectives of this research were to: (i) regenerate plants of Uruguayan dallisgrass through tissue culture, (ii) screen the regenerants for useful agronomic variation and evaluate their forage potential and nutritive value, and (iii) determine the genetic relatedness of the regenerants and their explant sources. In total, 178 plants, selected from 2372 regenerants in preliminary screening, were evaluated for forage nutritive value. Thirty-seven of these were planted into replicated field plot trials at two locations. None of these regenerants were superior to the Uruguayan biotype for forage nutritive value. However, two regenerants, 3440 and 3441, produced more forage than either the Uruguayan or common biotypes in evaluation tests for 3 years at one of the two locations. Data from AFLP analyses indicate genetic variation between two of the Uruguayan accessions and these two regenerants. This variation could account for the differences in forage yield between 3440 and 3441 and the Uruguayan accessions.

Introduction

Paspalum dilatatum Poir. (dallisgrass) is a perennial bunchgrass that provides summer forage for much of the higher-rainfall, subtropical coastal areas of New South Wales and Queensland in Australia and south-eastern USA (Burson and Watson 1995; Campbell 1999). It has also become a component of irrigated pastures in southern New South Wales, Western Australia (Callow *et al*. 2005) and northern Victoria (Lawson and Kelly 2007), where it has invaded temperate pastures. Dallisgrass is native to South America and was introduced into the USA sometime before 1842 (Chase 1929). The grass was first introduced into Australia in the early 1870s and has become naturalised in areas where it is particularly well adapted. Australia has been the world's largest producer and exporter of dallisgrass seed throughout most of the 20th century (Campbell 1999). Dallisgrass is primarily used as pasture forage and grows well in combination with legumes, such as white clover (*Trifolium repens* L.) (Bryan 1970; Whiteman 1980; Evers and Burson 2004). It is palatable and has the advantage of a longer growing season than most warm-season grasses (Holt 1956). Although dallisgrass is excellent forage for these subtropical regions, it is highly susceptible to ergot (*Claviceps paspali* Stevens and Hall) (Brown and Ranck 1915), which can cause livestock disorders. As with most warm-season grasses, forage quality of dallisgrass is distinctly lower than that of most temperate forages and its presence has had a negative effect on milk production on irrigated pastures in Victoria (Wales *et al*. 2000; Walker *et al*. 2001). However, early efforts to find superior genotypes of the genus *Paspalum* for south-eastern Queensland (Shaw *et al*. 1965) did not result in replacement of *Paspalum* pastures.

Common dallisgrass is an apomict (Bashaw and Holt 1958) with 50 chromosomes that associate as 20 bivalents and 10 univalents during meiosis (Bashaw and Forbes 1958). Efforts to improve the grass by breeding have not been successful because it is an obligate apomict with irregular meiosis (Tischler and Burson 1999; Venuto *et al*. 2003). Although apomixis can be useful in maintaining the genetic integrity of a species, it does not allow for the generation of variation that is essential for conventional plant improvement programs unless cross-compatible sexual plants are available. Efforts to

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regenerate common dallisgrass through tissue culture have been successful (Davies *et al*. 1986; Akashi and Adachi 1992; Burson and Tischler 1993). Somaclonal variation was observed in some of these regenerants, but none of them were superior to common dallisgrass (Davies *et al*. 1986; Davies and Cohen 1992; Burson and Tischler 1993; Tischler *et al*. 1993).

Besides common dallisgrass, there are at least seven other *P. dilatatum* biotypes. These are prostrate, Torres, Uruguaiana, Uruguayan, Vacaria, Virasoro and yellow-anthered (ssp. *flavescens*) (Evers and Burson 2004). Common, prostrate, Torres, Uruguaiana and Uruguayan are the better forage types and, among these, the Uruguayan biotype produced more forage and was more persistent than the others (Burson *et al*. 1991; Venuto *et al*. 2003). The Uruguayan biotype is an apomictic hexaploid with 60 chromosomes that pair during meiosis as 30 bivalents (Burson *et al*. 1991). However, like common dallisgrass, this biotype is susceptible to ergot and frequently produces poor quality seed with low viability (Burson *et al*. 1991; Tischler and Burson 1999). Use of tissue culture regeneration to produce somaclonal variants of Uruguayan biotypes has not been reported and may offer potential for improvement via this procedure.

Molecular markers provide an efficient tool to identify the limited amount of genetic variation typically produced through somaclonal regeneration. Comparative studies using marker techniques such as RFLP, RAPD, AFLP and SSR have shown that AFLP is the most efficient method to estimate genetic diversity because of its high reproducibility and polymorphism level, amenability to automation for high-throughput genotyping, and lack of requirement for sequence information (Vos *et al*. 1995; Powell *et al*. 1996; Russell *et al*. 1997; Pejic *et al*. 1998). In particular, AFLPs have been able to identify genetic variation between tissue culture-derived regenerants (Matthes *et al*. 2001). The objectives of this research were to: (i) regenerate selected hexaploid dallisgrass plants through tissue culture, (ii) screen individual regenerated plants for useful agronomic variation and evaluate their forage yield and nutritive value, and (iii) assess the genetic differences between the superior regenerants and the explant sources.

Materials and methods

Four accessions (PIs 404808, 404812, 404818 and 404820) of the Uruguayan biotype, a sexual yellow-anthered (ssp. *flavescens*) dallisgrass accession (PI 233053) and naturalised common dallisgrass were used as explant sources for regeneration purposes. The Uruguayan accessions and the yellow-anthered accession were all originally collected in Uruguay. Pieces of immature inflorescences were cultured on a Murashige and Skoog (1962) (MS) basal medium with 3% sucrose and 5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) to produce callus. When the callus developed embryonic areas, it was cultured on MS medium with 3% sucrose and 0.5 mg/L 2,4-D to initiate shoot development. Following shoot development, callus with embryos and plantlets was transferred to MS basal medium with 1.5% sucrose and no 2,4-D. Following root initiation and development, the plantlets were transferred to Oasis Wedge[1] growing medium and grown in a greenhouse. Dallisgrass plantlets were regenerated in 1994, 1995, 1996 and 1997. Once the plants were ~10 cm tall, they were transferred to pots and maintained in the greenhouse until planted in field plots. Field evaluation was conducted in two stages, involving initial screening of all surviving regenerants followed by a more thorough replicated evaluation of selected individual regenerants as presented in Table 1. Some regenerated plants were vegetatively propagated to produce ramets for testing at multiple locations.

Initial screening

Regenerants produced in 1994 and 1995 were initially screened at the Ben Hur Research Farm at Baton Rouge, Louisiana on a Sharkey clay loam (very-fine smectitic, thermic Chromic Epiaquerts) soil. In 1996 and 1997, the regenerants were screened at two locations: (i) the Baton Rouge site and (ii) the Rosepine Research Station, Rosepine, Louisiana on a Guyton silt loam (fine sandy-siliceous thermic Typic Glossaqualf). In 1994 and 1995, 174 and 751 regenerants, respectively, were transplanted as unreplicated individual plants on 1-m spacings. In 1996, 1247 different regenerants were transplanted and screened: 912 at Baton Rouge and 475 at Rosepine (140 of these were vegetatively cloned and planted at both locations). In 1997, 200 regenerants were cloned and transplanted at both locations. Vegetative cloning was done to provide plants for two replications of two ramets each at both sites. Preliminary screening consisted of visual assessment of plant growth and development. Seed was collected from selected plants during the growing season following transplanting.

At Baton Rouge, potentially superior plants from the original transplanting were selected and assessed for dry matter yield and forage nutritive value in the year following transplanting. Similar sampling of forage characteristics was done at Rosepine for only the 1997 regenerants. From 1994 through 1996, single-plant forage evaluations were taken on plant regrowth following seed collection and a mid-summer defoliation. Replicated samples were obtained from the 1997 regenerants. Whole plants were clipped at a height of 10 cm, dried at 60°C for 3 days, and weighed. Ground samples were analysed for forage nutritive value using near-infrared reflectance spectroscopy (NIRS) procedures. Dry matter yield, forage nutritive values, visual plant growth ratings and seed production traits were used to select those regenerants that would be evaluated further. Seed production characteristics of these plants have been reported elsewhere (Pitman *et al*. 2005).

Replicated field plot evaluation

Selected entries were vegetatively cloned and ramets were transplanted in replicated trials at the Baton Rouge and Rosepine sites. All trials were in randomised complete block designs with three replications. Controls used were common dallisgrass and the Uruguayan accessions PI 404808 and PI 404820. Management was similar among trials with 84 kg/ha of nitrogen applied at establishment and in the spring of each year. Forage was harvested from all plots by cutting at an average stubble height of 10 cm. Total fresh forage was weighed and a subsample collected and dried at 60°C for 3 days, to determine

[1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be available.

Table 1. Evaluation sequence for dallisgrass plants regenerated from tissue culture

Initial screening				Replicated evaluation		
Year regenerated	Number transplanted	Screening period	Number sampled[A]	Year transplanted	Evaluation period	Number evaluated
1994	174	1994–95	40	1996	1997–98	7
1995	751	1995–96	52	1997	1999–00	8
1996	1247	1996–97	34	1998	1999–01	10
1997	200	1997–98	52	1999	2000–01	12
Total	2372	1994–98	178		1997–01	37

[A]Number of plants evaluated for yield and forage nutritive value.

forage dry matter and calculate dry forage yield. Samples were then ground to pass through a 2-mm screen with a Wiley grinder and a subsample was saved for NIRS analysis.

Forage quality

NIRS spectral data were collected for each forage sample with a Model 6500 near-infrared reflectance spectrophotometer (NIRSystems). A library dataset was developed from samples analysed previously at the Louisiana State University Agricultural Center, Forage Quality Laboratory at the Southeast Research Station, Franklinton, Louisiana. The library file consisted of ~625 samples analysed for crude protein (CP), neutral detergent fibre (NDF), and *in vitro* true digestibility (IVTD) from previous research experiments. Reflectance data were related to the calibration data by using a modified partial least-squares regression procedure to develop the prediction equation (Shenk and Westerhaus 1991). Samples in the library file and from this experiment were analysed for CP colourimetrically (AOAC 1990), and NDF was measured using the methods described by Goering and Van Soest (1970), which were modified by excluding decalin. Additionally, 2 mL of a 2% (w/v) α-amylase solution was added at the beginning of the NDF procedure (Van Soest and Robertson 1980). *In vitro* true digestibility was measured using the methods described by Goering and Van Soest (1970).

DNA fingerprinting

Seedlings of the five plant introductions (PIs 404808, 404812, 404818, 404820 and 233053) that were used as tissue culture explant sources and two regenerants (3440 and 3441) were grown in pots in a greenhouse. Young leaves from each genotype were collected, lyophilised for 48 h, cut into small pieces, transferred along with three or four glass beads (4-mm diameter) into 1.5-mL tubes, and pulverised using a vortex genie. Genomic DNA was extracted as described in Williams and Ronald (1994).

AFLP reactions were performed as reported by Renganayaki *et al*. (2001). Seven *Eco*RI and *Mse*I primer combinations (E-ACC/M-CGC, E-CAT/M-CCA, E-CCA/M-CCA, E-CTA/M-CGA, E-CAG/M-CAT, E-CCT/M-CCT and E-CGT/M-CCG) were analysed using standard primers and adapters in the AFLP Analysis System I (Life Technologies, GIBCO BRL). PCR products were visualised using a LICOR model 4200 dual dye automated DNA sequencing system. Electrophoresis was performed for 3–3.5 h at a constant power of 40 W and a constant temperature of 47.5°C.

LICOR images were scored for the presence (1) and absence (0) of bands. Bands present in all seven entries were monomorphic, and the remaining polymorphic bands were used for fingerprinting analysis. Cluster and ordination analyses were performed using NTSYS-pc (Numerical Taxonomy for SYStematics) software version 2.0 (Rohlf 1997). The SIMQUAL function was used to calculate similarity coefficients between each pair of genotypes from Jaccard's coefficient ($J = a/[a + b + c]$), where a is the number of common bands (1, 1); b is the number of bands present in the first entry and absent in the second (1, 0); c is the number of bands absent in the first entry and present in the second (0, 1). The (0, 0) matches were not counted as informative because the lack of an AFLP band in two genotypes may not be due to a common evolutionary event. Cluster analysis was performed according to the unweighted pair group mean algorithm (UPGMA) within the SAHN function of NTSYS. The PCR, electrophoresis, gel staining and data analyses were replicated three times.

Results

Initial screening

Morphological variation among individuals was detected by observation and some appeared more vigorous and robust than others; however, much of this could be attributed to environmental effects. More distinct field variation among regenerants had been anticipated because of the amount of variation, especially detrimental, previously reported for common dallisgrass (Davies and Cohen 1992; Burson and Tischler 1993). Although plants were selected based on visual appearance of potentially superior forage traits, clipping results revealed a wide range in forage dry matter yield among selected regenerants during the first 3 years of evaluation (Table 2). Results indicate that no individual explant source consistently produced superior regenerants across all years. Less variation was observed for forage nutritive traits indicating less opportunity for selection of genotypes with improved nutritive values (Table 2).

Replication (ramets) of plants regenerated in 1997 was an attempt to more effectively screen for superior genotypes. Results (detailed data for individual regenerants not presented) of screening all of the 1997 regenerants, with the limited replication of individuals, produced 10-fold differences among entries in forage yield at individual locations. An entry by location interaction precluded identification of superior genotypes across locations. None of the five highest yielding individuals were the same for the two locations, although wide

ranges in responses were obtained. Again a superior explant source could not be identified (Table 2). Although responses for nutritive value shown in Table 2 appear to indicate substantial variation, this is an artefact of location effect. Differences in CP, NDF and IVTD were observed between locations, which probably reflect differences in plant maturity at harvest. Differences among entries within locations, however, were no greater than in the three previous years.

Replicated field plot evaluation

Although selection of regenerants for evaluation in these trials was independent of explant source, only regenerants from the Uruguayan accessions were selected. Annual dry matter production was compared only among individual entries within locations, since number of harvests per growing season and plant maturity at harvest differed between locations and among years. Dry matter production differed ($P < 0.05$) among entries in all trials except those at both locations evaluating the 1995 regenerants (Table 3). Although common dallisgrass was generally among the lower yielding entries, it differed ($P < 0.05$) from the two original Uruguayan accessions (standards) only in the comparison of regenerants from 1994 at Rosepine. In addition to these two Uruguayan accessions, three regenerants (designated 126, 188, and 205) produced more dry matter than common dallisgrass but not more than the two Uruguayan accessions at Rosepine. At Baton Rouge, two 1996 regenerants (3440 and 3441) produced more ($P < 0.05$) dry matter than common dallisgrass and the two Uruguayan accessions (Table 4). These two regenerants and two others (1645 and 3182) produced more forage than common dallisgrass but not more than either of the original Uruguayan accessions at Rosepine (Table 4). Although differences ($P < 0.05$) among entries were obtained at Rosepine for the 1997 regenerants, none were superior to either common or the two Uruguayan accessions. Most of the 1996 regenerants ranked similarly for yield at both locations (Table 4). At Baton Rouge, average yields of the1994 and 1996 regenerants were 33 and 58%, respectively, less than at Rosepine. However, the average yield of the 1995 regenerants was greater at Baton Rouge than Rosepine.

Forage nutritive values between locations were not compared because of confounding effects from differences in the number of harvests per year, harvest dates and plant maturity at harvest. Differences in CP among entries were not detected. However, differences among entries for both NDF ($P < 0.06$) and IVTD ($P < 0.001$) were observed only for the 1996 regenerants at Baton Rouge (Table 4), but were not observed for the regenerants, 3440 and 3441, that were superior for forage yield. Regenerant 1907 had the highest NDF that was superior ($P < 0.05$) to that of common dallisgrass and PI 404820, but not its explant source, PI 404808.

DNA fingerprinting

AFLP fingerprinting was done to assess the genetic diversity and relatedness of the five dallisgrass accessions used as explant sources and the two most productive regenerants, 3440 and 3441. A total of 174 bands were scored for seven primer combinations and 140 of these (80.5%) were polymorphic. This percentage of polymorphism is similar to estimates in rhodesgrass (*Chloris gayana* Kunth) (84%) (Ubi *et al*. 2003) and harding grass (*Phalaris aquatica* L.) (83.7%) (Mian *et al*. 2005). Pairwise genetic similarity values (Table 5) ranged from 0.33 (3441 *v*. PI 233053) to 0.94 (3441 *v*. 404808), with an average of 0.63. When the yellow-anthered biotype (PI 233053) is excluded, average pairwise genetic similarity between the hexaploid accessions and the regenerants is 0.73. A dendrogram produced by the UPGMA method (Fig. 1) clearly separated the

Table 2. Dry matter yield (DM), crude protein (CP), neutral detergent fibre (NDF) and *in vitro* true digestibility (IVTD) of selected dallisgrass tissue-culture regenerants during initial screening

Explant source	No. of regenerants evaluated	DM (g)		CP (g/kg)		NDF (g/kg)		IVTD (g/kg)	
		Mean ± s.d.	Range	Mean ± s.d.	Range	Mean ± s.d.	Range	Mean ± s.d.	Range
				1994 regenerants					
404808	10	120 ± 33.33	67–170	11 ± 0.46	11–12	70 ± 0.94	68–71	66 ± 1.04	65–68
404820	19	121 ± 23.63	80–158	11 ± 1.12	9–14	71 ± 0.52	70–72	65 ± 1.61	62–68
Common	11	102 ± 29.01	66–160	10 ± 0.75	9–11	71 ± 0.77	70–72	64 ± 1.40	61–66
				1995 regenerants					
404808	9	73 ± 18.71	54–114	9 ± 1.07	8–11	73 ± 1.31	70–74	62 ± 0.85	60–63
404820	41	62 ± 11.15	50–93	9 ± 0.67	7–11	73 ± 1.27	71–76	62 ± 1.43	58–65
Common	2	63 ± 8.49	57–69	10 ± 1.14	10–11	71 ± 1.10	70–72	61 ± 2.11	59–62
				1996 regenerants					
404808	20	27 ± 6.98	15–40	11 ± 0.77	10–12	69 ± 1.13	67–72	60 ± 1.66	57–63
404820	11	43 ± 11.27	23–57	11 ± 0.59	10–12	71 ± 0.87	69–72	59 ± 1.24	56–61
404808 or 404820[A]	3	39 ± 2.89	37–42	11 ± 1.32	10–12	70 ± 1.85	68–71	59 ± 2.52	57–62
				1997 regenerants					
404808	14	59 ± 16.29	40–89	11 ± 1.57	8–16	70 ± 2.13	64–74	69 ± 2.58	64–75
404812	5	66 ± 10.76	50–79	11 ± 0.46	11–12	70 ± 0.71	69–71	69 ± 0.93	68–71
404818	2	67 ± 6.19	63–72	10 ± 0.46	10–11	71 ± 0.33	71–71	68 ± 1.59	66–69
404820	31	59 ± 14.65	30–95	12 ± 1.00	11–16	70 ± 1.55	63–71	69 ± 1.92	65–72

[A]Exact identity lost.

entries into two major groups. The yellow-anthered biotype, PI 233053, was the only entry in one group and the remaining six entries were in the other group that divided into two subclusters. The first subcluster included two Uruguayan accessions, PI 404818 and PI 404820. The second subcluster consisted of two other Uruguayan accessions, PI 404808 and PI 404812, and the two regenerants, 3440 and 3441.

Discussion

Previous reports of variation from tissue-culture regeneration of common dallisgrass suggest that most phenotypic changes were undesirable. Davies and Cohen (1992) reported such deleterious or non-useful effects as dwarfism, change to a coarser plant type and changes in anther colour. Burson and Tischler (1993) noted a wide range among regenerants for leaf and inflorescence height along with the summation that essentially all changes were deleterious. However, we examined 2396 regenerants in the field and did not observe distinct deleterious changes, even though some differences in plant growth and rate of morphological development were discernable. The difference in the frequencies of regenerants with deleterious changes for these two biotypes could be a result of their chromosome compositions. Common dallisgrass is a pentaploid with the genome formula IIJJX (Burson 1983). Members of the X genome lack homologous chromosomes and, since all the genes on the chromosomes of this genome are in a hemizygous state, all recessive deleterious changes are expressed. The Uruguayan biotype has the genome formula IIJJXX (Burson 1991). Since the chromosomes of the X genome in this biotype have homologous members, recessive deleterious traits are not expressed unless the alleles on both homologues are recessive. Burson *et al*. (1991) concluded that most accessions of the Uruguayan biotype had potential to produce more forage than common dallisgrass; however, forage yield differences among biotypes and individual accessions were not consistent. Venuto *et al*. (2003) reported that both PI 404808 and PI 404820, among other hexaploid accessions, were consistently more productive than common dallisgrass in evaluations at the Baton Rouge location, even though such differences were not obtained at the Texas site. It appears that potential yield differences between a particular accession of the Uruguayan biotype and common dallisgrass may require appropriate environmental conditions for expression. Burson *et al*. (1991) reported small differences for *in vitro* digestibility among biotypes with common dallisgrass consistently near the median of the hexaploid biotypes evaluated. Venuto *et al*. (2003) reported that both PI 404808 and PI 404820 were higher in NDF than common dallisgrass in evaluations from sites in both Louisiana and Texas and higher *in vitro* digestibility only at the Texas

Table 3. Forage dry matter yield from replicated evaluations of dallisgrass tissue-culture regenerants at two locations in Louisiana
n.s., not significant

Entry type	No. of entries	Dry matter yield Baton Rouge[A]	Rosepine
1994 regenerants (g/plant)			
Hexaploid regenerants	7		
Mean		43	133.5
Range		19.3–60.3	90.5–183.2
Common dallisgrass	1	36.0	59.6
PI 404808	1	24.0	189.9
PI 404820	1	60.8	169.6
CV %		40.7	37.5
l.s.d. ($P = 0.05$)		36.8	73.7
1995 regenerants (kg/ha)			
Hexaploid regenerants	8		
Mean		7400	5020
Range		6790–8570	3610–6220
Common dallisgrass	1	6640	4640
PI 404808	1	8890	5620
PI 404820	1	7010	4880
CV %		16	45
l.s.d. ($P = 0.05$)		n.s.	n.s.
1996 regenerants (kg/ha)			
Hexaploid regenerants	10		
Mean		7779	13329
Range		5950–11 020	11 120–15 140
Common dallisgrass	1	7000	11 140
PI 404808	1	6940	13 880
PI 404820	1	7020	13 460
CV %		15	18
l.s.d. ($P = 0.05$)		1280	2760
1997 regenerants (kg/ha)			
Hexaploid regenerants	12		
Mean		–	20 050
Range		–	17 360–23 240
Common dallisgrass	1	–	20 350
PI 404808	1	–	19 500
PI 404820	1	–	21 160
CV (%)			12
l.s.d. ($P = 0.05$)			3280

[A]The trial at this location in 1997 was abandoned due to poor establishment.

Table 4. Dry matter yield (DM) of dallisgrass plants regenerated from tissue culture in 1996 and evaluated in replicated field plots in 1999, 2000 and 2001 at Baton Rouge and Rosepine, Louisiana, and neutral detergent fibre (NDF) and *in vitro* true digestibility (IVTD) from plants at Baton Rouge, Louisiana

Entry type	Baton Rouge NDF (g/kg)	Baton Rouge IVTD (g/kg)	Baton Rouge DM (kg/ha)	Rosepine DM (kg/ha)
Common	70.8	63.4	7000	11 140
PI 404808	71.3	62.1	6940	13 880
PI 404820	70.2	63.5	7020	13 460
1612	71.2	62.3	5950	11 120
2398	69.8	63.3	7030	11 970
1737	70.7	63.5	7430	12 310
1879	71.1	62.1	7680	12 320
1907	72.2	62.5	7080	12 920
1671	71.4	62.1	7620	13 690
1645	69.8	63.6	7940	14 040
3182	70.9	62.4	7620	14 650
3440	70.3	61.9	8420	15 140
3441	71.3	62.5	11 020	15 130
Mean	70.8	62.7	7430	13 200
CV (%)	3.2	4.3	15	18
l.s.d. ($P = 0.05$)	1.4[A]	1.7	1280	2760

[A]$P = 0.10$ for NDF.

Table 5. Genetic similarity coefficients between selected dallisgrass genotypes

	Genotypes						
	3441	PI404804	PI404812	3440	PI404818	PI404820	PI233053
3441	1						
PI404804	0.9432	1					
PI404812	0.9205	0.9318	1				
3440	0.8750	0.8409	0.8409	1			
PI404818	0.5114	0.5000	0.5000	0.5682	1		
PI404820	0.6818	0.6705	0.6477	0.7386	0.8068	1	
PI233053	0.3295	0.3864	0.3864	0.3409	0.3636	0.3750	1

location. In contrast, we did not detect differences between common dallisgrass and the accessions of the Uruguayan biotype for any measure of forage nutritive value.

As previously reported for the common dallisgrass biotype (Davies and Cohen 1992; Burson and Tischler 1993), very little of the variation generated through tissue culture will be useful for development of superior forage varieties. Forage nutritive value was particularly unresponsive to change through tissue-culture regeneration and results were similar to those previously reported for common dallisgrass (Davies and Cohen 1992). Differences obtained in measures of forage nutritive value between locations and among years primarily reflect stage of plant growth at harvest and environmental effects on plant growth and development. Such responses were also reported by Venuto *et al*. (2003) for the source genotypes (explants) of our regenerants. However, two regenerants that produced significantly more forage were identified. Although statistically different ($P < 0.05$) at only one location, these two regenerants ranked highest at the other location. Lack of a significant difference at the second location may reflect extent of variation among replications as suggested by a large l.s.d. value (Table 4). Identification of individual regenerants with potentially superior forage productivity and increased seed set (Pitman *et al*. 2005) provide hope for development of improved dallisgrass cultivars. Our results demonstrate that tissue culture regeneration has the potential to produce useful variation in the Uruguayan dallisgrass biotype and possibly other species that are difficult to improve through traditional plant breeding.

Genetic similarity between the hexaploid genotypes using AFLPs in this study was similar to that obtained using RAPDs in previous reports involving different dallisgrass biotypes (Casa *et al*. 2002; Miz and Souza-Chies 2006). AFLP fingerprinting revealed considerable genetic variation between the sexual yellow-anthered biotype (PI 233053) and the six hexaploid entries analysed, including the two regenerants (Fig. 1; Table 5). This was expected because both regenerants and the four Uruguayan accessions possess a complete genome that is not present in the yellow-anthered biotype. The yellow-anthered biotype is a tetraploid with the genomic composition of IIJJ (Burson 1978, 1983), whereas the regenerants and the Uruguayan accessions are hexaploids with a genome composition of IIJJXX (Burson 1991). The X genome has genes that govern traits that are specific to plants that possess this genome. Another factor that may contribute to the genetic differences between these two biotypes is their method of reproduction. The yellow-anthered biotype is sexual and the

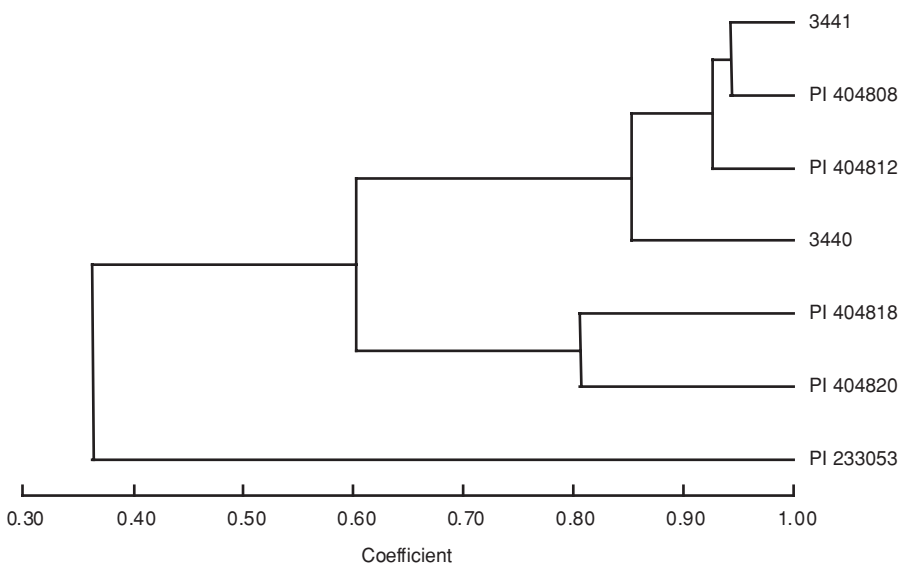

Fig. 1. Dendrogram showing the genetic similarity among five dallisgrass accessions and two regenerants as revealed by UPGMA cluster analysis based on AFLP data.

Uruguayan reproduces by apomixis. This should result in genetic differences in the I and J genomes of both biotypes. There was much less genetic variation between the Uruguayan accessions and the two regenerants (Fig. 1; Table 5). Both 3440 and 3441 were very similar to the Uruguayan accessions PI 404808 and PI 404812 (Table 5). PI 404808 was very likely the explant source of 3440, and 3441 originated from either PI 404808 or PI 404812. Data in Table 5 imply that regeneration through tissue culture produced modest genetic variation in the regenerants. When PI 404818 and 404820 are compared to PI 404808 and 404812, their similarity coefficients are not as close as expected (Table 5). Because all four accessions reproduce by apomixis and they were collected in the same geographical area, one would expect them to be more genetically similar. In another study investigating the genetic relatedness of these same four accessions, their coefficient similarities were in the 0.93 to 0.96 range (B. L. Burson, unpubl. data), which indicates the Uruguayan accessions may be genetically closer than indicated in this study. The reason for these differences is that seven highly polymorphic primer combinations were used in this study, whereas 25 were used in the earlier study without regard to level of polymorphism. If additional primer combinations had been used in this study, the similarity coefficients for the two regenerants and the four Uruguayan accessions would likely have been higher. Regardless, there appear to be true genetic differences between both PI 404808 and 404812 and the two regenerants. These differences could account for the variation in forage yield between 3440 and 3441 and the Uruguayan accessions.

Further multilocation evaluation of regenerants 3440 and 3441 is needed, since performance of these two entries was statistically superior only at the Baton Rouge location. This could indicate an important environmental interaction influencing yield of these regenerants compared to their explant sources. Data collected across multiple environments will provide information on genotype by environment responses and the agronomic application and usefulness of these superior regenerants.

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